"Hit" to Clinical Candidate

Discovery and Characterization of the Aminomethylcyclines

Corporate Overview

- Founded in 1996 by Drs. Walter Gilbert & Stuart Levy
- Located in Boston, MA
- Technologies from Dr. Levy's laboratory at Tufts University School of Medicine

Research Efforts

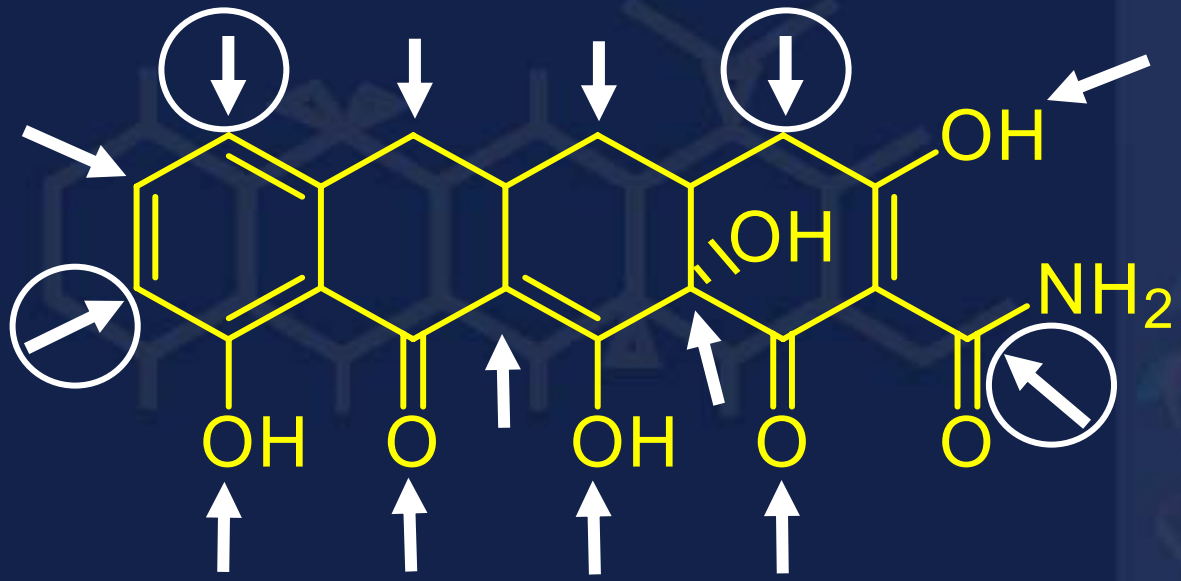
- Novel antibacterial tetracycline
  - PTK 0796
- Novel antiparasitic tetracycline
  - Malaria
- Novel *non-antibacterial* tetracyclines
  - Multiple Sclerosis
- Novel inhibitors of global regulation of virulence and efflux (MAR)

Why Tetracyclines?

- A well-known family of broad-spectrum antibiotics
- The family has been proven in the clinic with over 50 years of safety and proven efficacy
- Use has declined because of resistance
- Novel chemistry available to overcome resistance without sacrificing spectrum

Novel Chemistries Employed on Tetracyclines to Overcome Resistance

Proprietary Synthesis Approaches



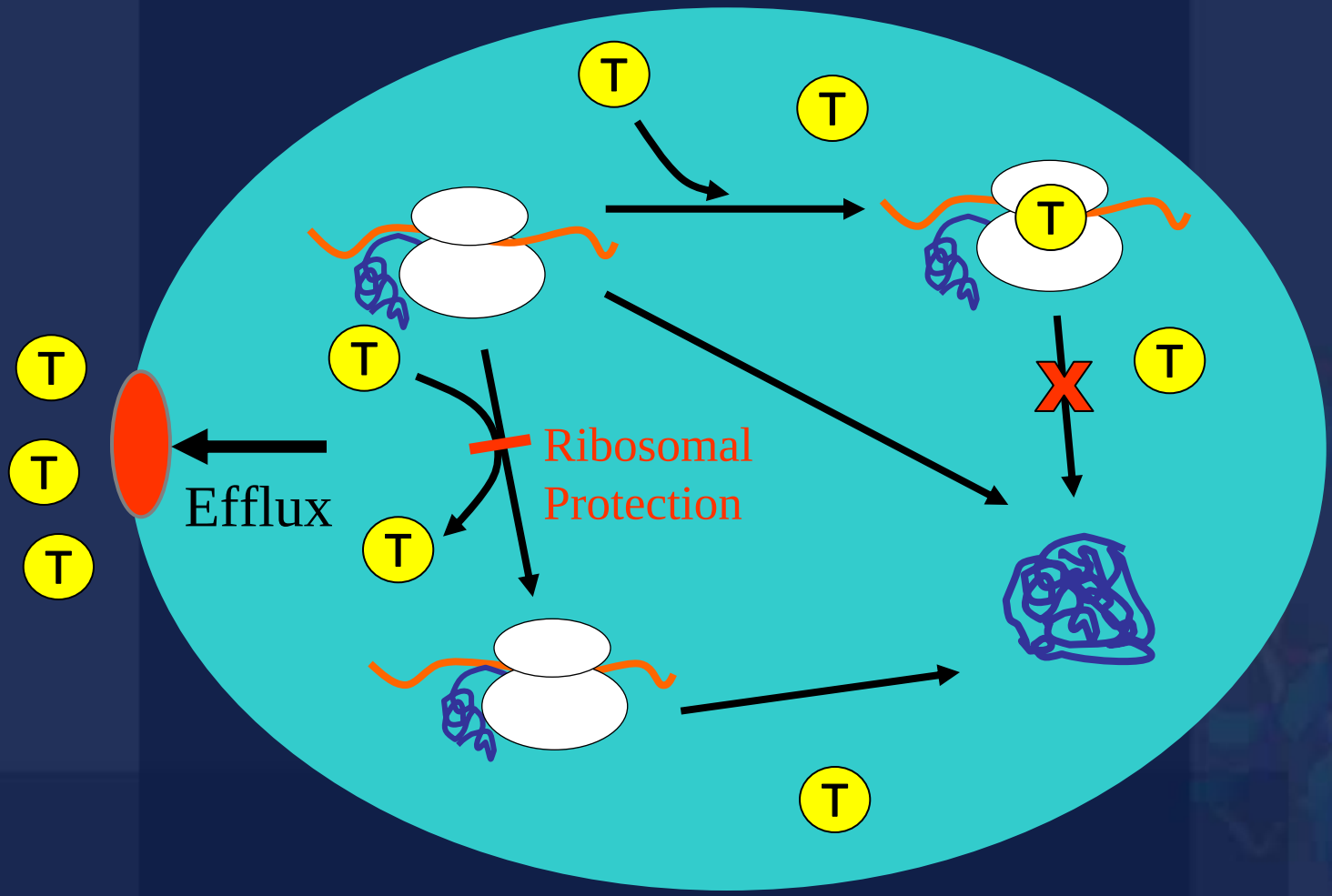
Proprietary Composition of Matter

= Historical sites of modification were limited

Original Target Profile

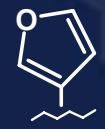
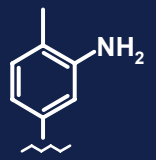
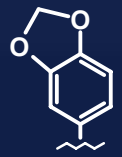
- Active in vitro against susceptible, tet R, and multi-resistant gram positive bacteria
  - Comparable to therapeutic comparators for susceptible isolates and superior for resistant isolates
- Effective in vivo against susceptible and resistant gram positive bacteria
  - Comparable to therapeutic comparators in standard models
- Once daily, intravenous delivery for hospitalized patients
- Safety and tolerability comparable or superior to standard tetracyclines

Tetracycline Resistance



Structure-Activity Relationships

- C7 modifications generally overcome efflux resistance
- C9 modifications generally overcome ribosome protection
- Polarity at C7 and C9 influence antibacterial potency
- Nonpolar modifications at C7/C9 imparts cytotoxicity/poor efficacy

Compound	R	MRSA	VRE	Ef	Spn
Doxycycline		8.0	16	4.0	4.0
P001075		1	1	≤0.06	≤0.06
P001036		0.25	2	≤0.06	0.13
P000642		0.5	0.5	≤0.06	0.25

Serum Interactions

Compound	No Serum	Human Serum	Mouse Serum
Doxycycline	8.0	8.0	8.0
P001075	1	>64	>64
P001036	0.25	>64	>64
P000642	0.5	>64	>64

MIC in μg/mL for **MRSA** in the absence and presence of human and mouse serum

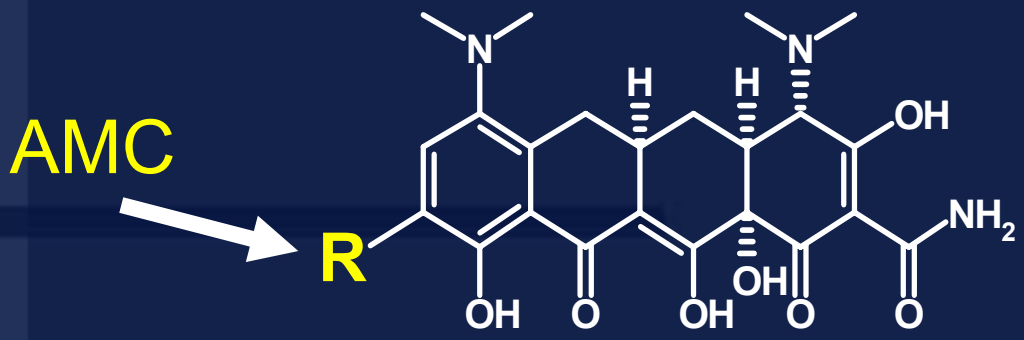
Cytotoxicity

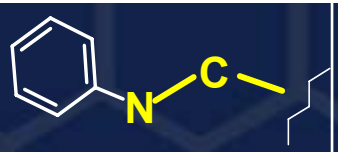
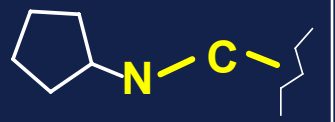
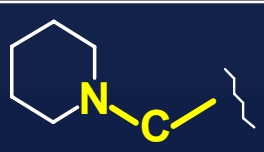
	Tox50 (μg/mL)	
Compound	**Cos-1**	**CHO-K1**
Doxycycline	>50	30
P001075	2.9	4.6
P001036	9.6	23
P000642	<1.6	<1.6

Efficacy

Compound	PD_{50} (mg/kg)
Doxycycline	2.3
P001075	>10
P001036	29
P000642	>10

PD_{50} (mg/kg) efficacy *in vivo* using a systemic *S. pneumoniae* (157E, Tet sensitive) infection model in mice. Compounds administered by single IV injection 1 hour post infection and survival monitored at day 7.



Compound	R	MRSA	VRE	Ef	Spn
P001221		0.5	1	0.25	≤0.06
P002352		0.5	0.5	1	≤0.06
P001207		0.5	0.5	1	≤0.06

Serum Interactions

Compound	No Serum	Human Serum	Mouse Serum
P001221	0.5	16	8
P002352	0.5	4	2
P001207	0.5	2	1

MIC in μg/mL for **MRSA** in the absence and presence of human and mouse serum

Cytotoxicity

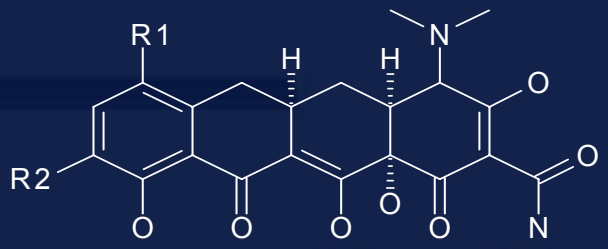
	Tox50 (μg/mL)	
Compound	**Cos-1**	**CHO-K1**
P001221	>100	>100
P002352	>100	>100
P001207	>100	>100

Efficacy

Compound	PD_{50} (mg/kg)
Doxycycline	2.3
P001221	0.63
P002352	0.43
P001207	0.58

PD_{50} (mg/kg) efficacy *in vivo* using a systemic *S. pneumoniae* (157E, Tet sensitive) infection model in mice. Compounds administered by single IV injection 1 hour post infection and survival monitored at day 7.

Structure-Activity



Organism	TetR	R1=H R2=H	R1=AMC R2=H	R1=H R2=AMC	R1=$N(CH_3)_2$ R2=AMC
S. aureus	none	<0.06	0.5	1.0	0.25
	Efflux	2.0	0.5	1.0	0.25
	TetM	2.0	2.0	2.0	0.25
E. faecalis	none	<0.06	1.0	1.0	0.5
	Efflux	1.0	0.5	0.5	0.25
	TetM	2.0	16	4.0	0.5
S. pneumoniae	none	<0.06	<0.06	<0.06	<0.06
	TetM	2.0	8.0	0.125	<0.06

Activity vs G+ Ribosome Protection

Organism	MIC (µg/ml)			
	AMC	Tet	Doxy	Mino
S. aureus ATCC 29213	0.13	0.25	<0.06	0.5
MRSA5 (TetM)	0.13	32	4.0	2.0
E. faecalis JH2-2	0.06	0.25	0.06	0.5
E. faecalis ATCC 29212 (TetM)	0.06	32	4.0	4.0
E. faecium 494 (TetL, TetM)	0.25	>64	8.0	16
S. pneumoniae 157E	0.06	0.25	<0.06	<0.06
S. pneumoniae 700905 (TetM)	0.06	64	4.0	8.0

Efflux Overcome *in vitro*

Organism	MIC (μg/ml)			
	AMC	Tet	Doxy	Mino
S. aureus RN450	0.25	<0.06	<0.06	0.5
S. aureus RN4250 (TetK)	0.25	64	4.0	1.0
E. faecalis JH2-2	1.0	0.25	0.06	0.5
E. faecalis 158 (TetL)	0.5	32	4.0	0.5
E. coli ML308-225	1.0	0.25	0.25	0.5
E. coli D1-299 (TetA)	2.0	32	8.0	2.0
E. coli D1-209 (TetBi)	1.0	8.0	2.0	2.0
E. coli pHCMI (TetBc)	1.0	64	16	4.0

Tetracycline Resistance Overcome *in vitro*

Organism	Tet Suscept (n)	MIC90 (μg/ml)			
		AMC	Tet	Mino	Doxy
S. aureus	S (35)	0.25	0.125	0.125	<0.06
	R (20)	0.5	64	8.0	8.0
E. faecalis	S (11)	0.5	0.25	0.5	<0.06
	R (20)	0.5	>64	16	16

Tetracycline Resistance Overcome *in vitro*

Organism	Tet Suscept (n)	MIC90 (μg/ml)			
		AMC	Tet	Mino	Doxy
E. faecium	S (8)	0.25	0.25	0.25	<0.06
	R (16)	0.5	64	16	8.0
S. pneumoniae	S (18)	0.125	0.125	0.25	0.125
	R (23)	<0.06	32	8.0	4.0

Tetracycline Resistance Overcome *in vitro*

Organism	Tet Suscept (n)	MIC90 (μg/ml)			
		AMC	Tet	Mino	Doxy
S. pyogenes	S (23)	0.25	0.125	0.5	<0.06
	R (7)	0.25	64	8.0	8.0
E. coli	S (17)	2.0	2.0	1.0	1.0
	R (6)	1.0	>64	16	64

In Vitro Activity vs Atypicals

Organism (n)	MIC 90 (µg/ml)		
	AMC	Mino	Doxy
C. pneumoniae (3)**	0.25	0.125	0.25
L. pneumophila (25)***	0.25	*NT*	1.0

**M. Hammersclag (max MIC); ***J. DuBois.

Activity vs Intracellular Pathogens

J774 macrophages infected with *Listeria*/*Salmonella* in-vitro

Organism	Compound	MIC (mg/l)	
		Extracellular	Intracellular
L.monocytogenes	**AMC**	**0.25**	**<0.03**
	Minocycline	**0.125**	**<0.03**
S. typhimurium	**AMC**	**8.0**	**1.0**
	Minocycline	**16**	**2.0**

Data from Bayer HealthCare

Resistance Development

- Single Step Resistance Mutations
  - Did not occur ($<10^{-9}$) in Tet S or TetR (efflux or protection) isolates of *S. aureus, E. coli, or E. faecalis*
- Multi-Step Resistance Mutations
  - Mutants of *S. aureus* (TetS and TetR efflux or protection) were not isolated after 10 serial passages

Tet Resistance Overcome *in vivo*

	Drug	MIC (μg/ml)	IV PD50 (mg/kg)
Susceptible	AMC	0.06	0.27
	Minocycline	<0.06	0.53
Resistant	AMC	0.06	0.14
	Minocycline	8.0	>100

Acute systemic *S. pneumoniae* 157E (sus) and *S. pneumoniae* 700905 (res). Animals infected IP at 100 x LD50. Treated once IV, 1 hour post-infection. Efficacy determined day 7 post infection.

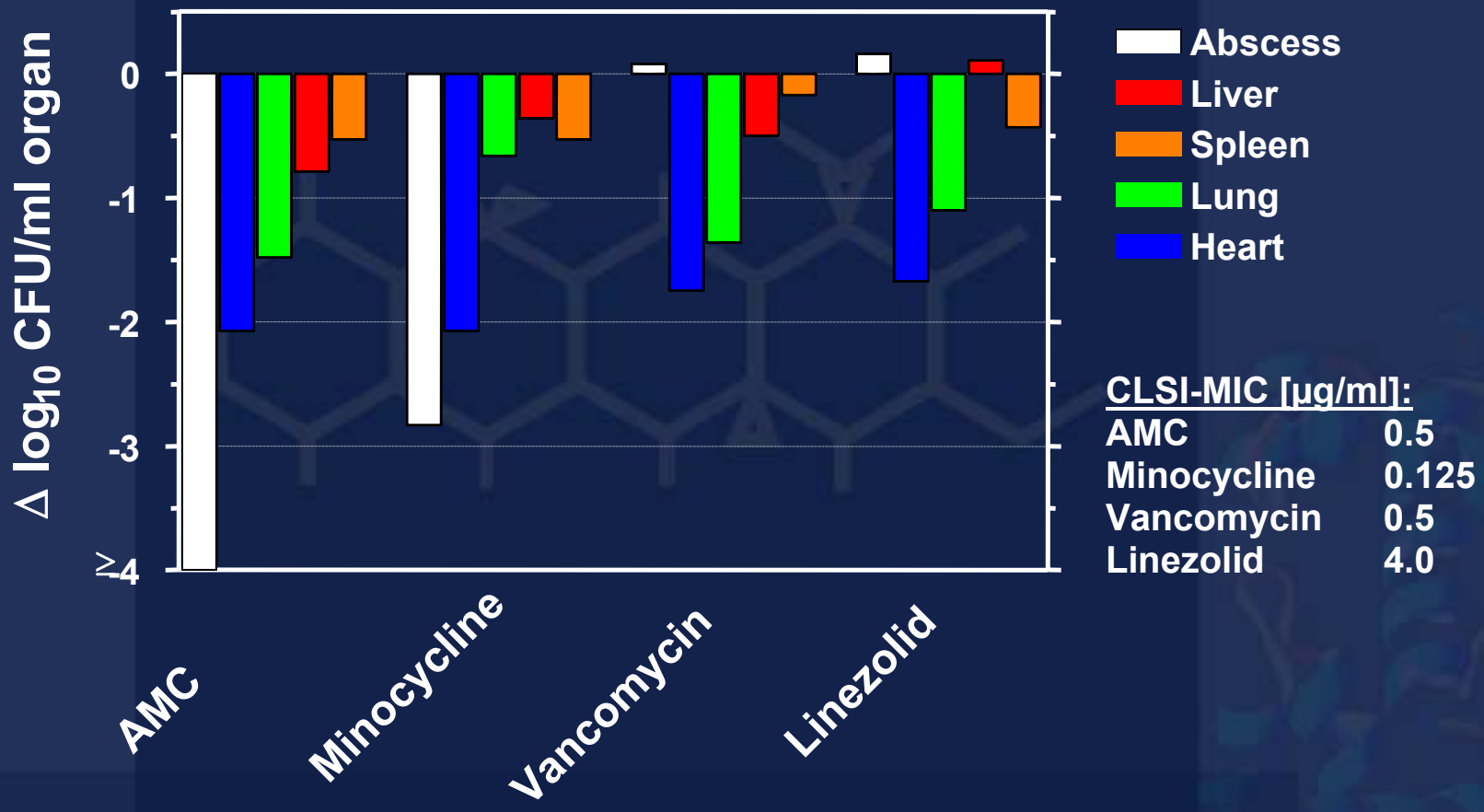
IV Efficacy in Mouse Models (1)

Model	Drug	MIC (μg/ml)	PD50 (mg/kg)
Systemic *S. aureus*	**AMC**	**0.5**	**0.4**
	Vancomycin	**0.5**	**0.4**
	Linezolid	**2.0**	**3.5**
Systemic VRE(neutropenic)	**AMC**	**0.12**	**5.0 (100%@15mg/kg)**
	Vancomycin	**>32**	**>50 (30%@50mg/kg)**
	Linezolid	**4.0**	**13.3 (60%@50mg/kg)**
Systemic *E. coli*	**AMC**	**2.0**	**9.0**
	Minocycline	**1.0**	**9.0**
	Ciprofloxacin	**0.6**	**4.2**
Acute Lung *S. pneumoniae* (neutropenic)	**AMC**	**<0.06**	**11.0**
	Vancomycin	**0.5**	**7.2**
	Linezolid	**1.0**	**>40**

IV Efficacy in Mouse Models (2)

Model	Drug	MIC (µg/ml)	ED50 (mg/kg)
Thigh Wound MRSA (neutropenic)	**AMC**	**0.5**	**5.9**
	Vancomycin	**1.0**	**30.4**
	Linezolid	**1.0**	**47.7**
Chronic Lung *S. pneumoniae*	**AMC**	**<0.06**	**7.4**
	Vancomycin	**0.5**	**>40**
	Linezolid	**1.0**	**>20**
Kidney *E. faecalis*	**AMC**	**0.5**	**4.5**
	Vancomycin	**2.0**	**70.3**
	Linezolid	**1.0**	**14.3**
Chronic LRTI *H. influenzae*	**AMC**	**1.0**	**4.7**
	Doxycycline	**0.25**	**18.6**
	Azithromycin	**1.0**	**31.6**
Subcutaneous Pouch *B. fragilis*	**AMC**	**<0.25**	**<12.5**
	Metronidazole	**0.5**	**<25**

Effectively Penetrates Abscesses in *S. aureus* Abscess Model

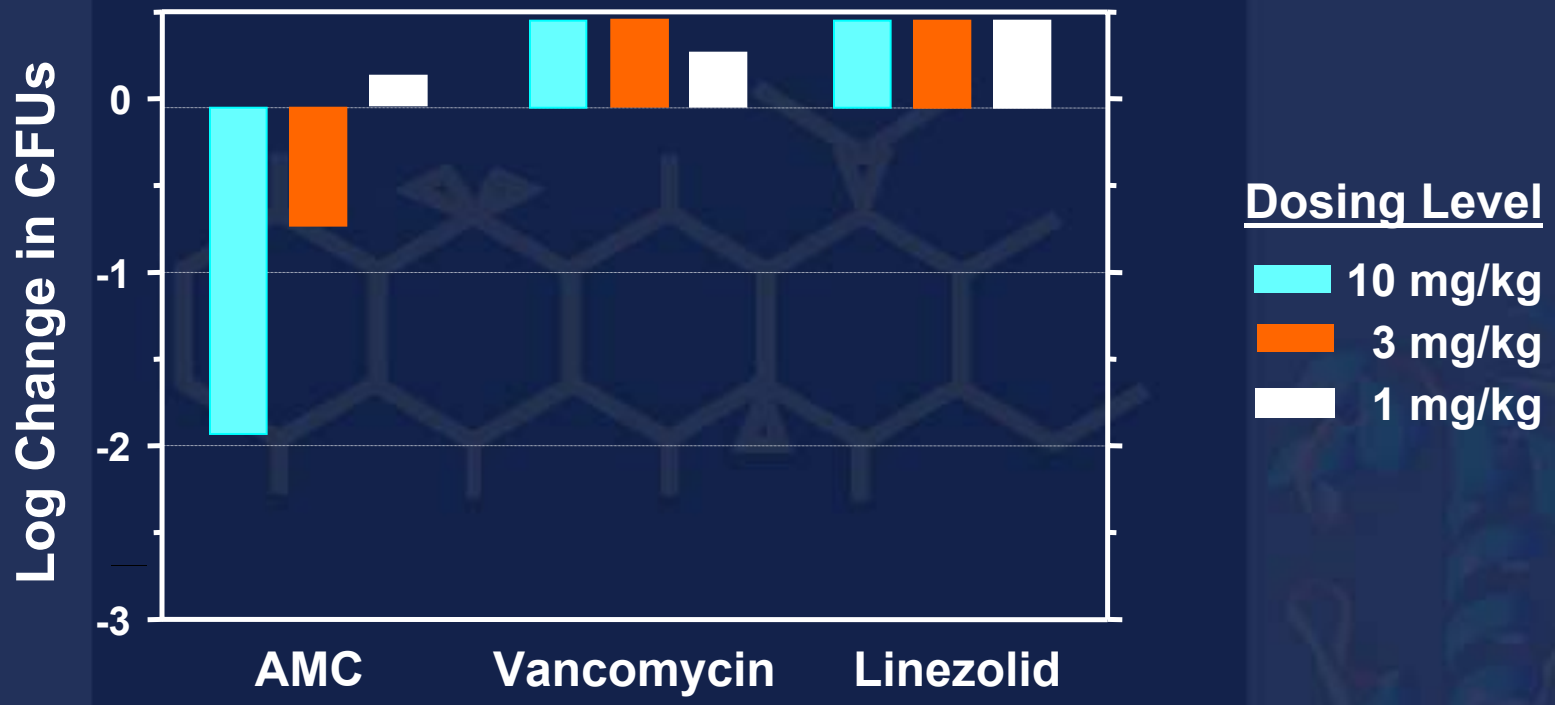


CLSI-MIC [µg/ml]:

AMC	0.5
Minocycline	0.125
Vancomycin	0.5
Linezolid	4.0

Gel-foam infected with MSSA strain is inserted in skin of mice causing chronic abscess to form. Treatment administered twice daily as IV dose with analysis at day 4 post infection (n=5 per treatment group). Graph shows log change in CFUs of homogenate versus controls. Study performed by Bayer.

Intracellular Efficacy Shown in *L. monocytogenes* Septicemia Model



Mouse model of sepsis via I.V. infection with clinical *Listeria monocytogenes* strain. Antibiotics delivered IV, 2 and 4 hours post-infection with analysis at day 5 post-infection (n=5 per treatment group). Graph shows log change in CFU/ml of spleen homogenate versus controls. Study performed by Bayer.

Single Dose Pharmacokinetics

Species (Organ)	Cmax (μg/ml)	AUC $_{0\text{-}24}$ (h·μg/ml)	t1/2 (h)	V_D (L/kg)
Mouse (plasma)	2.4	6.2	5.5	12.1
Rat (plasma)	1.7	5.6	3.8	7.4
Rat (lung)	20.7	33.7	3.9	ND
Rat (kidney)	26.9	59.0	3.6	ND
Monkey (plasma)	4.4	15.1	13.2	8.9

Doses: 10 mg/kg; ND, not determined.

Drug Metabolism/Elimination

- Drug metabolism
  - No induction or inhibition of CYP 450 enzymes
- Protein binding (monkey plasma)
  - 0-14% bound
  - Minocycline, doxycycline, tigecycline: 70-85% bound
- Elimination/Mass balance (using ^{3}H labeled drug)
  - 30% in urine
  - 24% in bile
  - 25% in feces (independent of bile)
  - Drug excreted unchanged; little metabolism

Target Profile

- Active *in vitro* against susceptible, tet R, and multi-resistant gram-positive, gram-negative, & atypical bacteria
  - Comparable to therapeutic comparators for susceptible isolates and superior for resistant isolates
- Effective *in vivo* against susceptible and resistant gram-positive, gram-negative, & atypical bacteria
  - Comparable to therapeutic comparators in standard models
- Once daily, intravenous and oral delivery
- Safety and tolerability comparable or superior to standard tetracyclines

Summary

- Aminomethylcyclines have:
  - Broad antibacterial spectrum including gram positive, gram negative, and atypical pathogens
  - Overcomes mechanisms of tetracycline and other antibiotic resistance
  - Microbiology, PK, ADME consistent with broad clinical utility
- PTK 0796/MK-2764 is the first AMC in clinical development

PARATEK PHARMACEUTICALS

A product-driven pharmaceuticals company developing novel therapies to *prevent*, *combat* and *cure* serious diseases